

# THERAPEUTICS AND MICROBIOTA

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**Abstract** – The gut microbiota is considered an essential “organ” due to its various functions and significance for human health, and its altered composition and function have been linked with various disease states of the host. Over the years, different strategies aimed at targeting the gut microbiota have emerged as promising tools for the prevention and management of several human disorders. This review summarizes notable findings from studies published between April 2021 and March 2022 evaluating the therapeutic potential of microbiota-modulatory agents, including prebiotics, probiotics, and synbiotics in intestinal and cardiometabolic diseases.

**Keywords:** Gut microbiota, Prebiotics, Probiotics, Synbiotics, Therapeutics, Intestinal disorders, Cardiometabolic, Chronic kidney disease.

## INTRODUCTION

In the last decade, human gut microbiota explorations have suggested great potential for manipulating the gut microbiota *via* different therapeutic agents to direct its composition and function towards health and to reduce the occurrence and severity of different human disorders. Some strategies like probiotics or fecal microbiota transplantation (FMT) rely on adding individual, several, or a whole consortium of living microbial organisms to exclude disease-causing microbes and provide health-promoting benefits<sup>1</sup>. Others aim to modulate the gut microbiota and its interaction with the host by using non-living agents like prebiotics that are utilized as substrates by specific groups within the microbiota to stimulate their growth and metabolic activity<sup>2</sup>. Moreover, bacteriocins and bacteriophages present another potential strategy to remove specific pathogens associated with the onset of a particular diseases<sup>1</sup>; however, their exploration as therapeutics in humans is still in its infancy.

Between April 2021 and March 2022, 1,528 papers examined the effects of microbiota-targeted therapeutics, including prebiotics, probiotics, and their blends (synbiotics) in different diseased states. Of these, 54 were results of clinical trials and meta-analyses of clinical trials performed with the adult subjects. This review focuses on the most notable findings from clinical studies evaluating the therapeutic potential of these microbiota-modulatory strategies in gastrointestinal and cardiometabolic-related disorders.

## GUT-MICROBIOTA MODULATORY THERAPEUTICS AND GASTROINTESTINAL TRACT-RELATED DISEASES

Probiotics, prebiotics, and synbiotics have been studied as a complementary or alternative therapy to conventional drug treatments in different gastrointestinal-related disorders (Table 1). They have been suggested to control symptoms and improve patients' quality of life by regu-



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**TABLE 1. SELECTED STUDIES EVALUATING THE EFFECTS OF PREBIOTIC/PROBIOTIC/SYMBIOTIC TREATMENTS IN DIFFERENT GASTROINTESTINAL AND CARDIOMETABOLIC-RELATED DISORDERS.**

Authors, year, country	Study design	Disease	Study subjects	Treatment, doses and duration	Main treatment effects
Wilson et al <sup>4</sup> 2021, United Kingdom	Open-label study, no CON	UC	13 patients with mildly active UC	GOS (2.8 g/d), 6 weeks	↑ normal stool % ↓ loose stool incidence, severity ≠ fecal calprotectin, SCFAs ↑ fecal <i>Bifidobacterium</i> , Christensenellaceae (subjects with SCCAI ≤ 2)
Hedin et al <sup>5</sup> 2021, United Kingdom	Open-label study, no CON	CD	19 patients with inactive CD (27.7 (6.9) y), 12 of their unaffected siblings	Oligofructose/inulin (15 g/day), 3 weeks	≠ fecal calprotectin (CD, siblings) ↑ fecal <i>Bifidobacteria</i> , <i>Bifidobacterium longum</i> (CD, siblings) ↑ <i>Bifidobacterium adolescentis</i> and <i>Roseburia spp.</i> (siblings); ↓ Intestinal permeability (CD) ↓ Blood T cell abundance (siblings)
Yang et al <sup>6</sup> 2021, China	DB, P, RCT	IBS	55 patients with chronic diarrhea TRT: n = 28; 52.7 ± 9.1 y PLB: n = 27; 47.4 ± 12.8 y	<i>Lactiplantibacillus plantarum</i> CCFM1143 (3.52 × 10 <sup>9</sup> CFU/day) or maltodextrin, 4 weeks	↓ fecal <i>Bacteroides</i> , <i>Eggerthella</i> ↑ <i>Akkermansia</i> , <i>Terrisporobacter</i> , <i>Anaerostipes</i> ↑ fecal acetic and propionic acids (vs. baseline) improved chronic diarrhea symptoms (vs. PLB) ≠ MTL, TNF, IL-6, 5-HT, VIP (vs. PLB)
Chen et al <sup>9</sup> 2021, Taiwan	DB, P, RCT	<i>H. pylori</i> infection	40 subjects with moderate-high bacterial loads of <i>H. pylori</i> TRT: n = 20; 49.0 ± 13.3 y PLB: n = 20; 47.0 ± 14.6 y	<i>Lactobacillus acidophilus</i> and <i>Lactocaseibacillus rhamnosus</i> (6 × 10 <sup>9</sup> CFU/day) or placebo 4 weeks	↓ bacterial load of <i>H. pylori</i> (vs. PLB) 0% eradication rate for <i>H. pylori</i> (in both groups) ≠ α, β-diversity
Yuan et al <sup>7</sup> 2021, China	DB, P, RCT	<i>H. pylori</i> infection	95 <i>H. pylori</i> -positive subjects QT: n = 34; 26.32 ± 2.53 y TRT: n = 30; 26.5 ± 2.6 y QT+TRT: n = 31; 26.0 ± 2.5 y	<i>Bifidobacterium infantis</i> , <i>Lactobacillus acidophilus</i> , <i>Enterococcus faecalis</i> (each at 4.5 × 10 <sup>6</sup> CFU/day), <i>Bacillus cereus</i> (4.5 × 10 <sup>5</sup> CFU/day) with or without QT ( <i>bismuth,esomeprazole, amoxicillin, clarithromycin</i> ), 2 weeks	QT+TRT: ↓ gastric <i>Fusobacterium</i> , <i>Campylobacter</i> , <i>Proteobacteria</i> , <i>Mycoplasma</i> , <i>Leptotrichia</i> , ↑ <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> , <i>Eubacterium ventriosum</i> gastric microbial diversity closer to <i>H. pylori</i> -negative subjects compared to QT. TRT: ≠ <i>H. pylori</i> inhibition ↑ gastric <i>Fusobacterium</i> , ↓ <i>Bifidobacterium</i> , <i>Faecalibacterium</i>

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TABLE 1 (CONTINUED). SELECTED STUDIES EVALUATING THE EFFECTS OF PREBIOTIC/PROBIOTIC/SYMBIOTIC TREATMENTS IN DIFFERENT GASTROINTESTINAL AND CARDIOMETABOLIC-RELATED DISORDERS.

Authors, year, country	Study design	Disease	Study subjects	Treatment, doses and duration	Main treatment effects
Guillemard et al <sup>8</sup> 2021, Germany	DB, P, RCT	<i>H. pylori</i> infection	136 adults under 2-week <i>H. pylori</i> treatment TRT: n= 68; 42.1 (10.1) CON: n=68; 42.6 (11.3)	Test drink with <i>Lactocaseibacillus paracasei</i> CNCM I-1518 and I-3689, <i>L. rhamnosus</i> CNCM I-3690 and 4 yogurt strains or Control drink, 4 weeks (2 during, 2 after <i>H. pylori</i> treatment: <i>pantoprazole</i> , <i>clarithromycin</i> , <i>amoxicillin</i> )	↓ intra-subject $\beta$ -diversity distance from baseline (vs. CON) ↓ fecal <i>Escherichia-Shigella</i> , <i>Klebsiella</i> (vs. CON) ↑ major fecal SCFA, valerate (vs. CON)
Neyrinck et al <sup>12</sup> 2021, Belgium	SB, P, RCT	Obesity	24 obese patients from FOOD4GUT cohort TRT: n = 12 PLB: n = 12	Native inulin (16 g/d) or maltodextrin (16 g/d) with dietary advice to consume inulin-rich or -poor vegetables and follow dietary caloric restriction, 12 weeks	↑ fecal <i>Bifidobacterium</i> , ↓ fecal calprotectin, ↑ fecal tauroconjugated/ free bile acids ratio, ≠ fecal zonulin, SCFAs, ↑ fecal rumenic acid (all vs. baseline)
Rodriguez et al <sup>11</sup> 2022, Belgium	SB, P, RCT	Obesity	61 obese patients from FOOD4GUT cohort TRT: n = 30 (n = 14 with increased PA) PLB: n = 31 (n = 19 with increased PA)	Native inulin (16 g/d) or maltodextrin (16 g/d) with dietary advice to consume inulin-rich or -poor vegetables and follow dietary caloric restriction, 12 weeks	Higher ↓ body weight and BMI in TRT with increased PA vs. TRT ↓ plasma TC, gGT, fasting insulin, HOMA-IR only in TRT+ PA ↑ fecal <i>Bifidobacterium</i> , <i>Cantaniibacterium</i> and <i>Dialister</i> only in TRT+ PA
Watanabe et al <sup>10</sup> 2021, Japan	DB, P, RCT	Obesity	38 obesity-prone subjects TRT: n = 20; 45.2 (9.5) y PLB: n=18; 43.4 (11.7) y	1-kestose (10 g/d) or maltodextrin (10 g/d), 12 weeks	↓ fasting serum insulin (vs. PLB) ↑ fecal <i>Bifidobacterium</i> (vs. PLB), ↓ <i>Blautia</i> , <i>Sellimonas</i> (vs. PLB)

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Authors, year, country	Study design	Disease	Study subjects	Treatment, doses and duration	Main treatment effects
Wang et al <sup>19</sup> 2022, China	DB, P, RCT	T2D	365 newly diagnosed T2D patients TRT: n = 92; 52.11 ± 8.74 y TRT+BRB: n = 98; 52.9 ± 9.1 y BRB: n=84; 52.07 ± 10.81 y PLB: n=91; 52.56 ± 9.44 y	Multi-strain probiotic ( <i>Bifidobacterium longum</i> CGMCC No. 2107; <i>B. breve</i> CGMCC No. 6402; <i>Lactococcus gasseri</i> CGMCC No. 10758; <i>Lactocaseibacillus rhamnosus</i> CNCM I-4474; <i>Ligilactobacillus salivarius</i> CGMCC No. 6403; <i>Lactobacillus crispatus</i> CGMCC No. 6406; <i>Lactiplantibacillus plantarum</i> ; CGMCC No. 1258; <i>Limosilactobacillus fermentum</i> CGMCC No. 6407; <i>Lactocaseibacillus casei</i> CNCM I-4458; ≥ 50 billion CFU/day) or probiotic placebo, with BBR (1.2 g/day) or BRB placebo, 12 weeks	TRT+BBR improved postprandial TC and LDLc more than BBR or TRT alone and reduced multiple postprandial lipidomic metabolites at 3 months follow-up ↑ fecal <i>Bifidobacterium breve</i> levels after TRT+BBR and ↓ after BBR alone
Ming et al <sup>18</sup> 2021, China	DB, P, RCT	T2D	297 newly diagnosed patients with hyperglycemia BRB: n = 49; 53.28 ± 9.87 y TRT: n = 100; 54.16 ± 9.10 y TRT+BBR: n = 49; 53.36 ± 9.49 y PLB: n = 99; 52.73 ± 9.35 y	<i>Bifidobacterium adolescentis</i> capsules (2x10 <sup>8</sup> CFU/day) or probiotic placebo with BRB tablets (1 g/day) or BRB placebo, 16 weeks	↓ fasting plasma glucose, 2-h postprandial plasma glucose in TRT+BRB, BRB (vs. PLB) ↓ HbA1c (vs. PLB) TRT+BRB, BRB more alterations in fecal microbiota from the baseline (vs. TRT or PLB)

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Authors, year, country	Study design	Disease	Study subjects	Treatment, doses and duration	Main treatment effects
Hata et al <sup>17</sup> 2022, Japan	Open-label, single-arm trial	T2D	40 T2D patients using metformin (64.0 ± 9.4 y)	<i>Bifidobacterium bifidum</i> G9-1 (Biofermin tablets- 12 mg of bifidobacteria, 3x6 tablets/day), 10 weeks	↓ GI symptom rating scale total score ↓ GI diarrhea and constipation subscale scores ≠ HbA1c, glucose levels ↓ fecal Sutterella ≠ α, β-diversity
Mohamad Nor et al <sup>21</sup> 2021, Malaysia	DB, P, RCT	NFLD	39 ultrasound diagnosed NFLD patients TRT: n = 17; 54.70 (10.19) y PLB: n = 22; 52.47 (16.73) y	Probiotic mixture ( <i>Lactobacillus acidophilus</i> BCMC 12,130, <i>Lactocaseibacillus casei</i> subsp. BCMC 12,313, <i>Lactobacillus lactis</i> BCMC 12,451 <i>Bifidobacterium bifidum</i> BCMC 02290, <i>B. infantis</i> BCMC 02129 and <i>B. longum</i> BCMC 02120 – 60 x 10 <sup>9</sup> CFU/day) or placebo, 6 months	≠ hepatic steatosis, fibrosis levels (vs. PLB) ≠ ALT, TC, triglycerides, and fasting glucose (vs. PLB), ↓ CD8+T lymphocytes expression in small intestinal villi and ZO-1 expression in crypt area only in PLB
Ebrahim et al <sup>26</sup> 2022, South Africa	SB, P, RCT	CKD	45 non-diabetic, non-dialysis, stage 3-5 CKD patients TRT: n = 30; CON: n = 29;	β-glucan prebiotic fiber supplement (13.5 g/day) or CKD-advised diet only, 14 weeks	↓ plasma free IxS, pCS and pCG (vs. CON) ≠ urea, creatinine, eGFR (vs. CON), <i>Bacteroides 2</i> to <i>Prevotella</i> enterotype shift trend (vs. baseline)
Armani et al <sup>25</sup> 2022, Brazil	DB, P, RCT	CKD	46 non-diabetic, non-dialysis, stage 3-5 CKD patients TRT: n=23; 61.96±11.4 y PLB: n=23; 53.46±16 y	FOS (12 g/day) or maltodextrin, 12 weeks	↓ plasma IL-6 (vs. baseline) ≠ serum total pCS, IxS (vs. baseline) ≠ FMD, PWV (vs. PLB) In subjects with less severe endothelial dysfunction FMD remained stable in TRT and ↓ in PLB

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Authors, year, country	Study design	Disease	Study subjects	Treatment, doses and duration	Main treatment effects
Cosola et al <sup>24</sup> 2021, Italy	SB, P, RCT, pilot	CKD	23 non-diabetic, non-dialysis stage 3b-4 CKD patients, 24 healthy controls TRT: n = 12 CKD, n = 12 healthy; PLB: n = 10 CKD, n = 15 healthy;	1 NATUREN G <sup>®</sup> synbiotic/day: <i>Lactocaseibacillus Casei</i> LC4P1 (2.4 × 10 <sup>9</sup> CFU), <i>Bifidobacterium Animalis</i> BLC1 (2.4 × 10 <sup>10</sup> CFU), FOS (2.5 g), inulin (2.5 g) and natural antioxidants (quercetin-0.064 g, resveratrol-0.023 g, proanthocyanidins-0.013 g) or placebo for 8 weeks	↓ serum free IxS (vs. PLB in CKD) ≠ serum total, free pCS, total IxS (vs. PLB in CKD) ↓ small intestine permeability (vs. baseline, CKD) Improved abdominal pain, constipation symptoms (vs. baseline, CKD)
McFarlane et al <sup>27</sup> 2021, Australia	DB, P, RCT	CKD	56 non-dialysis, stage 3-4 CKD patients TRT: n=28; PLB: n=28;	High-resistant starch fiber (20 g/day) + probiotic (4.5 × 10 <sup>11</sup> CFU/day <i>Lactobacillus acidophilus</i> , <i>L. delbrueckii subsp. Bulgaricus</i> , <i>Lactiplantibacillus plantarum</i> , <i>Lactocaseibacillus paracasei</i> , <i>Bifidobacteria breve</i> , <i>B. longum</i> , <i>B. infantis</i> , <i>Streptococcus thermophilus</i> ) or waxy maize powder + maltodextrin (all at 1/2 dose first 2 weeks), 12 months	↑ fecal <i>Bifidobacterium</i> , <i>Blautia spp.</i> ↓ eGFR (vs. PLB) ↑ serum creatinine (vs. PLB) ≠ serum total, free IxS, pCS (vs. PLB) ≠ serum lipids, BP (vs. PLB)
He et al <sup>28</sup> 2022, China	DB, CO, RCT	End-stage CKD	16 continuous ambulatory peritoneal dialysis patients 37.67 ± 11.65 y	Inulin/oligofructose (10g/day) or maltodextrin, 12 weeks	↓ serum UA (vs. PLB) daily UA renal excretion ↑ fecal UA degradation (vs. baseline), ↑ fecal Firmicutes/ Bacteroidetes ratio (vs. baseline) ↑ purine-degrading species (vs. baseline)

≠, non-significant change; ↑, significant increase; ↓, significant reduction; 5-HT, 5-hydroxytryptamine; ALT, alanine aminotransferase; BBR, berberine treatment; BP, blood pressure; BMI, body mass index; CD, Crohn's disease; CFU, colony forming units; CKD, chronic kidney disease; CO, crossover design; CON, control; DB, double-blind; eGFR, estimated glomerular filtration rate; gGT, gamma-glutamyl transferase, FMD, flow-mediated dilatation; FOS, fructooligosaccharide; GI, gastrointestinal; HbA1c, glycosylated hemoglobin; IAA, indoxyl 3-acetic acid; IBS, Irritable bowel syndrome; IxS, indoxyl-sulfate; IL-6, interleukin-6; LDL-c, low-density lipoprotein cholesterol; MLT, motilin; P, parallel design; PA, physical activity; pCG, p-cresyl glucuronide; pCS, p-cresyl sulfate; PLB, placebo; PWV, pulse wave velocity; RCT, randomized controlled trial; SB, single-blind; SCCAI, simple clinical colitis activity index; TC, total cholesterol; TNF, tumor necrosis factor alpha; TRT, treatment group; UA, uric acid; VIP, vasoactive intestine peptide; QT, bismuth-containing quadruple therapy; ZO-1, zona occludens-1 protein.

lating the impaired gut microbiota consortia observed in these conditions. The therapeutic potential of these agents in inflammatory bowel disease (IBD), a relapsing multifactorial disease comprising ulcerative colitis (UC) and Crohn's disease (CD), was evaluated in a meta-analysis of 32 randomized controlled trials (RCTs)<sup>3</sup>. The authors found that these therapeutics considerably increased the number of beneficial intestinal bacteria (particularly *Bifidobacterium*), induced or maintained IBD remission and lowered UC disease activity index whilst not affecting IBD recurrence. Subgroup analyses showed that combining these agents with conventional therapies was more effective in reducing these parameters than traditional treatments alone, while synbiotic treatment seemed to be more effective than prebiotics and probiotics alone. Additionally, the study suggested that probiotics containing *Bifidobacterium*, *Lactobacillus*, or more than one bacterial strain were more effective as IBD therapeutics and proposed doses from  $10^{10}$  to  $10^{12}$  colony forming units (CFU)/day as reference dose.

The severity of inflammation and disease activity has also been proposed to influence the effectiveness of microbiota-targeted therapeutics in IBD. An open-label study examining the effect of consuming 2.8 g/day of galactooligosaccharide for 6 weeks in patients with mildly active UC showed normalized bowel function and no significant impact on inflammation, short chain fatty acid (SCFA) production, or previously shown changes in *Bifidobacterium*. However, prebiotics increased *Bifidobacterium* and *Christensenellaceae* only in subjects with less active UC at baseline (simple clinical colitis activity index  $\leq 2$ )<sup>4</sup>. Similarly, a pilot study<sup>5</sup> evaluating the effect of a 3-week oligofructose/inulin supplementation (15 g/day) in subjects with inactive CD and their unaffected siblings showed that, while having comparable levels at baseline, siblings better responded to prebiotic and had a considerably larger increase in *Bifidobacteria*, *B. adolescentis*, and *Roseburia* spp. Moreover, in unaffected siblings, *B. adolescentis* concentration was associated with decreased blood T cell numbers. Therefore, together with previously published findings<sup>3</sup>, the data could suggest that the microbiota-modulatory efficacy of these prebiotics decreases going from healthy, at-risk subjects to those with inactive and active IBD, indicating their potential in IBD primary prevention and treatment in a less inflamed gut.

The effect of probiotics on chronic diarrhea, associated with different intestinal disorders like irritable bowel syndrome and functional diarrhea, was also evaluated. Yang et al<sup>6</sup> showed that intake of  $3.52 \times 10^9$  CFU/day of *Lactiplantibacillus plantarum* CCFM1143 (previously *Lactobacillus plantarum*) for 4 weeks was effective in managing chronic diarrhea symptoms in patients compared to placebo (maltodextrin). Moreover, prebiotic consumption decreased the abundance of *Bacteroides* and *Eggerthella*, increased the abundance of beneficial species (*Akkermansia*, *Terrisporobacter*, and *Anaerostipes*), and stimulated acetic and propionic acid production, altogether suggesting the potency of this strain to improve the microbiota imbalance and clinical symptoms in functional bowel disorders.

The therapeutic potential of probiotics alone or in combination with standard treatments has also been evaluated in *Helicobacter pylori* infection<sup>7-9</sup>. One study<sup>8</sup> showed that consumption of a probiotic-containing drink (fermented milk with *Lactocaseibacillus paracasei* CNCM I-1518 and I-3689, *L. rhamnosus* CNCM I-3690, and four yogurt strains) for 28 days did not affect antibiotic-associated diarrhea and gastrointestinal symptoms in subjects under 2-week triple eradication treatment (proton pump inhibitor (PPI) and two antibiotics). However, it induced faster gut microbiota recovery after *H. pylori* eradication, reducing the abundance of potentially pathogenic bacteria (e.g., *Escherichia-Shigella* and *Klebsiella*) and increasing fecal SCFA generation compared to the control drink. Another study evaluated the therapeutic effects of a probiotic (*Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, and *Bacillus cereus*), provided alone or in combination with quadruple eradication therapy (PPI, bismuth, and two antibiotics) for 2 weeks, on gastric microbiota recovery in *H. pylori*-infected individuals<sup>7</sup>. Results showed that 2 months after treatment, the quadruple therapy did not restore gastric microbiota of *H. pylori*-positive subjects to an uninfected state; however, adjuvant probiotic therapy contributed to its recovery by improving microbial diversity, reducing the abundance of potentially harmful bacteria (e.g., *Fusobacterium*, *Campylobacter* and *Proteobacteria*) and increasing the beneficial bacteria (e.g., *Lachnospiraceae*, *Ruminococcaceae*, *Eubacterium ventriosum*). By contrast, probiotic monotherapy was ineffective in *H. pylori* abolition and failed to restore gastric microbiota, with observed alterations in microbiota structure, increased putative pathogenic bacteria, and no induction of beneficial bacteria growth (*Bifidobacterium* and *Lactobacillus*). Similarly, a 4 week-long probiotics monotherapy with *Lactobacillus acidophilus*

and *Lactocaseibacillus rhamnosus*, although reduced the bacterial load of *H. pylori*, failed at its eradication and did not induce significant changes in gut microbiota diversity and composition<sup>9</sup>. Therefore, these findings together support the use of probiotics as a complementary therapy for *H. pylori* eradication in infected subjects while not recommending their use as the sole treatment.

## GUT-MICROBIOTA TARGETED THERAPEUTICS IN OBESITY, TYPE 2 DIABETES, AND NON-ALCOHOLIC FATTY LIVER DISEASE

A few studies<sup>10-12</sup> evaluated the potential of prebiotics to modulate gut microbiota and induce metabolic improvements in obesity, which is a major risk factor for developing different cardiometabolic disorders that are linked with alterations in gut microbiota composition and function. In a study by Neyrinck et al<sup>12</sup>, obese subjects were supplemented for 3 months with 16 g/day of native inulin or maltodextrin and advised to consume inulin-rich or -poor vegetables and follow dietary caloric restriction (Table 1). Results showed the effectiveness of inulin in reducing gut inflammation, measured by fecal calprotectin, which correlated with reduced *Actinomyces* and *Erysipelotrichaceae* (UCG003) observed in the inulin-treated group. Although not altering SCFA production, inulin raised fecal levels of rumenic acid. This conjugated linoleic acid, with previously reported anti-obesogenic and anti-inflammatory effects<sup>13</sup>, can be produced by bifidobacteria *in vitro*<sup>14</sup>, and accordingly, its increase correlated with higher *Bifidobacterium* abundance in the inulin-treated group. In another study<sup>11</sup>, these authors showed that the success of the same inulin supplementation in obese subjects was influenced by physical activity, which enhanced beneficial metabolic effects of inulin, including improved glucose tolerance, body mass index (BMI), plasma total cholesterol (TC), liver enzymes as well as the expansion of *Bifidobacterium*, *Cantaniibacterium* and *Dialister* bacteria. Ameliorations in glucose metabolism were also observed following a 12-week consumption of 1-kestose (fructooligosaccharide-type prebiotic) in obesity-prone subjects and were accompanied by changes in microbiota composition, including increased fecal *Bifidobacterium* and reduced *Blautia* levels<sup>10</sup>.

Probiotics were also suggested as a promising therapeutic option in managing obesity and its associated comorbidities like T2D and non-alcoholic fatty liver disease (NAFLD) (Table 1). In a meta-analysis of 26 RCTs with 1720 overweight/obese subjects<sup>15</sup>, probiotics significantly reduced BMI, body weight, waist circumference, fat mass, abdominal fat area, insulin, TC, low-density lipoprotein cholesterol (LDL-c), and tumor necrosis factor alpha compared to control groups. Doses of 10<sup>10</sup> CFU or higher and administrations equal to or longer than 8 weeks were reported as effective in improving body adiposity parameters in these individuals. Furthermore, another meta-analysis of 26 RCTs in patients with T2D showed the effectiveness of prebiotics in reducing fasting blood glucose, which was more pronounced in subjects with poorly controlled diabetes<sup>16</sup>. In a recent exploratory study with T2D patients using metformin, the administration of *Bifidobacterium bifidum* G9-1 for 10 weeks was shown to modulate gut microbiota and improve gastrointestinal symptoms associated with this antidiabetic drug, suggesting the benefit of combining prebiotics with traditional drug treatments in T2D<sup>17</sup>. Moreover, coadministration of *Bifidobacterium* supplement and berberine (a plant-derived chemical with antidiabetic and hypolipidemic effects) for 18 weeks was more effective in reducing fasting plasma glucose and 2-h postprandial plasma glucose as well as modulating gut microbiota in newly diagnosed hyperglycemia patients than each of these treatments individually<sup>18</sup>. Similarly, the synergistic effect of berberine and a multi-strain probiotic was reported in lowering postprandial TC and LDL-c concentrations as well as reducing the levels of several postprandial lipidomic metabolites in newly diagnosed hyperglycemia patients after a 3 month-treatment<sup>19</sup>. These effects were associated with changes in fecal *Bifidobacterium breve* levels that were reduced by berberine and increased following its coadministration with prebiotic. Further mechanistic explorations showed that in *B. breve*, berberine induces the expression of genes involved in lipid import and mobilization, which could result in lower intestinal lipid absorption of the host. The findings suggested that coadministration with the probiotic is needed to recover *B. breve* levels and thereby enhance berberine effects on microbial lipid mobilization, translating into a more pronounced hypolipidemic effect, which further supported the combined use of probiotics with other T2D treatment options.

Studies<sup>20-23</sup> from the preceding year also evaluated the therapeutic action of prebiotics in NAFLD, which is associated with obesity, T2D, and dyslipidemia and marked by gut microbiota dysbiosis. More specifically, a meta-analysis of 9 RCTs including 352 NAFLD patients showed that prebiotic treatment significantly reduced serum levels of alanine aminotransferase (ALT), aspartate transaminase (AST), and TC compared to the control group<sup>20</sup>. In these patients, three or more combined probiotics and administrations longer than 12 weeks were reported to also significantly lower BMI. Similarly, a network meta-analysis of 22 RCTs comparing the effects of probiotics, prebiotics, and their blends in 1301 NAFLD patients suggested that prebiotics were the most effective in reducing BMI, ALT, AST, and LDL-c<sup>22</sup>. By contrast, a recent RCT failed to report the effects on different clinical markers of NAFLD, including ALT, TC, triglyceride, and fasting glucose levels, following a 6-month-long supplementation with a probiotic containing strains of 6 different *Lactobacillus* and *Bifidobacterium* species<sup>21</sup>. However, it indicated this probiotic's capacity to act locally in the small intestine, stabilizing mucosal immune function and protecting these subjects from increased intestinal permeability.

## GUT MICROBIOTA TARGETED MODULATION IN CHRONIC KIDNEY DISEASE

Modulation of the gut microbiota has been proposed as a therapeutic target in controlling chronic kidney disease (CKD). In this disorder, the intestinal microbiota overproduces proteolytic uremic toxins, such as p-cresyl sulfate (pCS), p-glucuronide (pCG), and indoxyl sulfate (IxS), whose increased passage to the systemic circulation, due to the impaired gut integrity, and reduced kidney elimination contribute to overall disease progression<sup>24</sup>. Moreover, these toxins have been associated with inflammation, endothelial dysfunction, and cardiovascular disorders in CKD patients<sup>25</sup>. A few studies<sup>24-27</sup> have evaluated the effectiveness of prebiotic or synbiotic supplementation in modulating gut microbiota, reducing uremic toxin production, and improving clinical manifestations in CKD patients and provided inconsistent results (Table 1). Ebrahim et al<sup>26</sup> showed that consuming 13.5 g/day of  $\beta$ -glucan supplement for 14 weeks resulted in decreased plasma levels of free pCS, IxS, and pCG, without changes in kidney function in stage 3-5 CKD patients. The authors also observed a trend towards a shift from *Bacteroides 2* to *Prevotella* enterotype in the prebiotic group with no changes in dietary intake, suggesting a potentially favorable change in gut microbiota composition induced by  $\beta$ -glucan. By contrast, only a slight but non-significant decrease in pCS was observed in CKD patients following a 3-month intervention with prebiotic fructooligosaccharide (12 g/day), accompanied by a significant reduction in interleukin 6 levels and preserved endothelial function in CKD patients with a less severe endothelial dysfunction<sup>25</sup>. No changes in free pCS were also reported by Cosola et al<sup>24</sup> in stage 3b-4 CKD patients following a 2-month supplementation with NATUREN G<sup>®</sup>, a synbiotic composed of fructooligosaccharide, inulin, polyphenolic antioxidants, *Lactocaseibacillus casei* (previously *Lactobacillus casei*), and *Bifidobacterium animalis* BLC1. This synbiotic, however, significantly reduced free IxS levels, intestinal permeability, abdominal pain, and constipation syndrome in the same subjects. Interestingly, these effects were not seen in healthy volunteers, implying the specificity of this synbiotic's action in the CKD. No changes in either IxS and pCs levels were detectable in stage 3-4 CKD patients following a 1-year supplementation with synbiotic containing high-resistant starch and strains of different *Lactobacillus*, *Lactiplantibacillus*, *Lactocaseibacillus*, *Bifidobacteria*, and *Streptococcus* species, while there was a significant increase in some acetate and butyrate-producing bacteria (e.g., *Bifidobacterium* and *Blautia* spp.) in the stool<sup>27</sup>. Although this study showed good feasibility of long-term synbiotic therapy and no changes in gastrointestinal symptoms, the observed reduction in kidney function, evidenced by reduced estimated glomerular filtration rate and increased serum creatinine levels compared to placebo, raises caution and warrants further examination.

The therapeutic potential of prebiotics in the final stage of CKD was also evaluated. Results from a recent crossover RCT showed that treatment with 10 g/day of inulin/oligofructose prebiotic for 12 weeks significantly reduced serum uric acid levels and stimulated fecal uric acid degradation in peritoneal dialysis patients compared to placebo (maltodextrin)<sup>28</sup>. Moreover, the prebiotic induced an increase in the Firmicutes/Bacteroides ratio and levels of some purine-degrading species, suggesting that by modulating gut microbiota composition and its uricolytic activity, this inulin-type prebiotic may exert the uric acid-lowering effect and be used as an additional therapeutic option in end-stage renal disease.

## CONCLUSIONS

Over the years, accumulating evidence has shown that the gut microbiota plays an important role in the pathogenesis of different intestinal and cardiometabolic disorders<sup>29-31</sup>, and therapeutic options aimed at targeting gut microbiota and restoring its balance show great potential in the prevention and treatment of these disorders. During the preceding year, the majority of clinical studies suggested some therapeutic potential of using prebiotics, probiotics, or synbiotics in these human disorders; still, validation of these results in larger cohorts is recommended. Some research failed to report the beneficial effects of these microbiota-modulatory agents<sup>21,32,33</sup>, possibly due to specific prebiotic type, probiotic strain, formulation, dosage, and treatment duration selected for the investigation. Studies also suggested that an individual's lifestyle, age, disease severity, as well as the use of traditional medications may influence its response to microbiota-modulatory agents<sup>4,7,11,17</sup>. Therefore, more well-designed clinical trials, taking into account all these aspects, are needed to confirm the effectiveness of prebiotic/probiotic/synbiotic in the prevention and treatment of these human disorders.

### Conflict of Interest

The authors declare no conflict of interest.

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